REMARKS

The specification has been amended to delete the last four words of paragraph [0073].

The specification has been further amended to set forth the current status of U.S. patent application Serial No. 09/318,136 referenced in paragraph [0099].

Paragraph [0100] has been amended to include text found in the '136 application which had been incorporated by reference, and paragraph [0116] has been amended to include text found in U.S. Patent No. 5,565,355 which had been incorporated by reference. A Verified Statement concerning this amendatory material accompanies this Amendment.

Paragraph [0119] has been amended to correct the reference to preceding Examples.

It is submitted that none of these amendments to the specification is new matter and their entry is requested.

Claims 7, 28, 34, 37, 38, 41 and 46-62 have been canceled by the present amendment.

Claims 1, 25 and 39 have been amended to specify the composition of the liquid culture medium as comprising inorganic nutrients, amino acids, vitamins, carbohydrate source and an eradicant. Support for this language can be found in the Examples which describe the liquid culture media useful in the present invention.

Claims 8 and 42 have been amended to be consistent with the cancellation of claims 7 and 41.

Claim 12 has been amended to clarify the language of the claim noting that it is the washed cells that are cultured for selection.

Claim 19 has been amended to specify that washed cells are further contacted with an *Agrobacterium* eradicant during selection.

Claim 20 has been amended to more clearly specify the nature of the contacting of claim 19.

Claims 21-23 have been amended to clarify that the layer contains the eradicant.

Claim 25 has been further amended to delete the eradication step and to incorporate the selection step of claim 28.

Claims 29 and 35 have been amended to specify that washed cells are further contacted with an *Agrobacterium* eradicant during selection.

Claims 30 and 36 have been amended to more clearly specify the nature of the contacting of claim 29 and 36, respectively.

Claim 39 has been further amended to specify a definition for the term "minimizing damage." Support for this amendment can be found at page 8, line 34.

New claims 82-119 have been added. Support for new claims 82, 85, 88, 91 can be found in Example 1. Support for new claims 83, 84, 86, 87, 89, 90, 92, 93, 116 and 117 can be found in Example 6. Support for claims 94, 95, 101, 102, 118 and 119 can be found in Example 5. Support for claims 96-100 can be found in original claims 13-17. Support for claims 103-115 can be found in the original claims, as well as in the support noted for the above amendments and new claims.

It is submitted that these amendments do not constitute new matter and their entry is requested.

The Examiner objected to paragraph [0073] of the specification. It is believed that the amendment of this paragraph obviates this objection.

The Examiner rejected claims 1-9, 11-43 and 45 under 35 U.S.C. § 112, first paragraph for failing to comply with the written description requirement. The Examiner contends that the term "osmoticum" constitutes new matter. Although Applicants do not agree with the Examiner's rejection because all of the liquid culture media described in the specification contain an osmoticum, they have nevertheless deleted this term. It is believed that this amendment obviates this rejection, and its withdrawal is requested.

The Examiner rejected claims 1-9 and 11-38 under 35 U.S.C. § 112, first paragraph for lack of enablement. The Examiner contends that the specification does not provide enablement for a method that:

(a) does not use a culture medium for washing that comprises inorganic salts, vitamins, amino acids, inositol, casein hydrolysate, sucrose and auxin, cytokinin or ABA;

(b) does not include an eradication step;

(c) does include ABA in any of the steps; and

(d) does not wash the cells between *Agrobacterium* infection step, *Agrobacterium* eradication step, selection step or growing the transformed pine cells into transformed somatic embryo step wherein the cells or embryos are collected on a support membrane placed over medium comprising the constituents.

It is submitted that the Examiner is in error with respect to certain aspects of this rejection. It is further submitted that the above amendments and following remarks demonstrate that the specification fully enables the claimed subject matter.

First, Applicants have amended the claims to specify that the liquid culture medium used for washing comprises inorganic nutrients, vitamins, amino acids, a carbohydrate source and an *Agrobacterium* eradicant. Applicants note that inositol is a vitamin and that casein hydrolysate is a source of amino acids. In addition, Applicants note that the specification teaches that and liquid culture medium can be used for washing the transformed pine cells and that hormones and ABA are not required to be present in the liquid culture medium used for washing. The specification teaches that any liquid culture medium can be used to wash the transformed pine cells. *See*, page 9, paragraph [0031]. The specification provides two examples of liquid culture media used for washing the transformed pine cells.

The composition of a first liquid culture medium is set forth in Example 1 in which the wash medium is liquid DCR_4. *See*, page 23, paragraph [0063]. DCR_4 comprises inorganic nutrients, vitamins, amino acids and a carbohydrate source as shown in Table 2. DCR_4 also comprises hormones. It does not contain ABA. The composition of a second liquid culture medium is set forth in Example 6 in which the wash medium is the maintenance medium of U.S. 5,565,355 without gelling agent. *See*, page 49, paragraph [0122]. in conjunction with amended paragraph [0116]. The maintenance medium of the '355 patent comprises inorganic nutrients, vitamins, amino acids and a carbohydrate source. This maintenance medium does not contain hormones and does not contain ABA. In addition, paragraph [0069] on page 24 indicates that the wash medium is a culture medium, i.e., it could be any culture medium, such as also disclosed in paragraph [0118] on page 48. Culture

media are known in the art to contain inorganic nutrients, vitamins, amino acids and a carbohydrate source, and may optionally contain hormones. Thus, the specification clearly teaches that the liquid culture medium for washing transformed pine cells only needs to contain inorganic nutrients, vitamins, amino acids and a carbohydrate source and can be any culture medium.

As shown in these same examples, the wash medium further contains an eradicant to eradicate the Agrobacterium. Consequently, the "liquid culture medium" has been defined in the claims to comprise inorganic nutrients, vitamins, amino acids, a carbohydrate source and an *Agrobacterium* eradicant. In view of these clear teachings in the specification, it is submitted that the claims in which the liquid culture medium for washing that comprises inorganic nutrients, vitamins, amino acids, a carbohydrate source and an *Agrobacterium* eradicant are fully enabled.

Second, as previously noted, Applicants have amended the claims to specify that the liquid culture medium used for washing includes an *Agrobacterium* eradicant. Thus, the claims specify an *Agrobacterium* eradication step which is concomitant with the washing. Further eradication can occur during the selection step as shown in Examples 3 and 5. This aspect of the invention has been set forth in several dependent claims. Example 3 shows that this aspect of the invention may be beneficial for some species of pine of the subgenus *Pinus*. In view of the amendment to the claims, it is submitted that the specification fully enables the claimed method in which an eradicant is contained in the wash medium, thus imparting an eradication step to the claimed method.

Third, the specification demonstrates that ABA is not required in the steps of the method, although the use of ABA in certain steps may be preferred, for all members of the subgenus *Pinus*. The specification clearly teaches that ABA may be beneficial for certain species of pine of the subgenus *Pinus*, but is not necessary for all species. For example, the specification discloses that it is preferred to use ABA in the selection medium for *P. taeda* and certain hybrids as shown in Examples 7 and 8. These Examples demonstrate that ABA may be preferred in other media, but is not required, because it gave either neutral results or beneficial results. However, Example 6 demonstrates that ABA does not need to be present in the selection medium, let alone any of the other media, for *P. radiata*. In addition, paragraph [0048] on page 16, specifically noted by the

Examiner, only teaches that the presence of ABA is useful for certain species of pine of the subgenus *Pinus*, namely the Southern yellow pines, and does not teach that ABA is required for all members of the subgenus *Pinus*. The specification provides guidance for the use of ABA in selection medium, as well as in other media. Namely, the use of ABA is preferred, especially for those species of pine of the subgenus *Pinus* which have low rates of recovery of viable transformed cells using media without the ABA. Thus, it is submitted that the specification fully enables the claimed method which does not require the use of ABA in the selection medium or in any other media.

Fourth, the specification clearly teaches that support membranes are not required for each of the steps of the method, although their use may be preferred. For example, Example 4 shows that the use of support membranes is not required for the selection of transformed pine cells. In addition, Example 6 shows that support membranes are not required, although preferred for ease of manipulation, for preparing plants of the species *P. radiata*. In addition, the Examples show that washings are not required between each of the steps, i.e., none of the Examples describe any washing steps other than the washing after the co-cultivation of pine cells with *Agrobacterium* in the manner to minimize damage to the transformed pine cells. Thus, it is submitted that the specification fully enables the claimed invention which does not require the use of support membranes and which does not have washing steps between each step of the method set forth in the claims.

In addition, Applicants note that paragraph [0118] on page 48 teaches that any nutrient media that is commonly used for *Pinus* somatic embryogenesis is suitable for use in the method of the invention. The specification has demonstrated the use of different nutrient media in the method of the invention. Thus, it is submitted that the specification fully enables the use of various nutrient media in the claimed method.

In view of the above amendments and remarks, it is submitted that the specification fully enables the claimed subject matter. Withdrawal of this rejection is requested.

The Examiner rejected claims 46-48 under 35 U.S.C. § 102(b) as being anticipated by Handley et al. (US 5,491,090). Although Applicants do not agree with the Examiner's rejection of

all of these claims, they have nevertheless canceled these claims to expedite the allowance of the application. Withdrawal of this rejection is requested.

The Examiner rejected claims 39-43 and 45 under 35 U.S.C. § 103(a) as being unpatentable over Levee et al. (*Molecular Breeding* 5:429-440, 1999) in view of Handley et al. The Examiner's primary basis for this rejection appears to relate to the lack of a definition of the term "minimizing damage" leading to a broad reading of the claim. Applicants have amended claim 39 to specify the definition for this term. This amendment is consistent with the language found in claim 1. Levee et al. does not disclose a method to minimize damage to transformed cells of the subgenus *Pinus* as presently claimed for the same reasons as detailed in previous amendments with respect to claim 1, as well as with respect to differences between the soft pine of Levee et al. and the hard pines of the present invention as detailed in the previous amendments and supported by the Rule 132 Declarations previously submitted. Handley et al. does not add anything to the teachings of Levee et al. Thus, it is submitted that this cited art does not render claims 39-43 and 45 obvious.

In view of the above amendments and remarks, it is submitted that the claimed invention is not obvious from the teachings of Levee et al. in view of Handley et al. Withdrawal of this rejection is requested.

The Examiner rejected claims 46-51 under 35 U.S.C. § 103(a) as being unpatentable over Handley et al. Although Applicants do not agree with the Examiner's rejection of these claims or the characterization of the teachings of the cited reference, they have nevertheless canceled these claims to expedite the allowance of the application. Withdrawal of this rejection is requested.

The Examiner rejected claims 52-57 under 35 U.S.C. § 103(a) as being unpatentable over Levee et al. Although Applicants do not agree with the Examiner's rejection of these claims or the characterization of the teachings of the cited reference, they have nevertheless canceled these claims to expedite the allowance of the application. Withdrawal of this rejection is requested.

The Examiner rejected claims 58-62 under 35 U.S.C. § 103(a) as being unpatentable over Levee et al. Although Applicants do not agree with the Examiner's rejection of these claims or the

characterization of the teachings of the cited reference, they have nevertheless canceled these claims to expedite the allowance of the application. Withdrawal of this rejection is requested.

In view of the above amendments and remarks, and in conjunction with the remarks made in the previous amendments and previously filed Rule 132 Declarations, it is believed that the claims satisfy the requirements of the patent statutes and are patentable over the prior art. Reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

ROTHWELL, FIGG, ERNST & MANBECK, p.c.

By _____

Jeffrey L. Ihnen
Registration No. 28,957
Attorney for Applicant
1425 K Street, N.W., Suite 800
Washington, D.C. 20005
phone: 202-783-6040
fax: 202-783-6031

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